CLAIMS:

1. A method of staining targeted chromosomal material based upon nucleic acid sequence employing nucleic acid probes wherein said targeted chromosomal material is in the vicinity of a suspected genetic rearrangement.

2. A method according to Claim 1 wherein the targeted chromosomal material is one or more metaphase and/or interphase chromosomes, or one or more regions thereof.

3. A method according to Claim 2 wherein the chromosomal material is human and selected from chromosomes 1 through 22, X and Y.

4. A method according to Claim 3 wherein the chromosomal material is of fetal cells.

5. A method according to Claim 4 wherein the fetal cells have been separated from maternal blood.

6. A method according to Claim 1 wherein said nucleic acid probes comprise heterogeneous mixtures of labeled nucleic acid fragments, wherein a substantial fraction of the sequences of the labeled nucleic acid fragments are substantially complementary to sites on chromosomal material that are targeted and are substantially free of nucleic acid sequences having hybridization capacity to sites on chromosomal material that is not targeted.

7. A method according to Claim 1 wherein the genetic rearrangement is selected from the group consisting of translocations, inversions, insertions, amplifications and deletions.

8. A method according to Claim 1 wherein the genetic rearrangement is associated with a disease.

9. A method according to Claim 8 wherein the genetic rearrangement is associated with cancer.

10. A method according to Claim 9 wherein the genetic rearrangement is diagnostic for chronic myelogenous leukemia (CML).

11. A method according to Claim 10 wherein the genetic rearrangement is selected from the group consisting of translocations, deletions, amplifications and insertions.

12. A method according to Claim 11 wherein said nucleic acid probes are homologous to nucleic acid sequences in the vicinity of the translocation breakpoint regions of chromosomal regions 9q34 and 22q11 associated with CML.

13. A method according to Claim 12 wherein said nucleic acid probes produce a staining pattern which is distinctively altered when the BCR-ABL fusion characteristic of CML occurs.

14. A method according to Claim 12 wherein signals from said nucleic acid probes when hybridized to said nucleic acid sequences produce staining patterns as represented in Figure 11, sections b-e, inclusively.

15. A method according to Claim 13 wherein the proximity of and/or other characteristics of signals of said staining pattern indicate whether said BCR-ABL fusion is present.

16. A method according to Claim 15 wherein the portion of the probe to the BCR region is labeled/visualized in one manner and the portion of the probe to the ABL region is labeled/visualized in another manner so that when the BCR-ABL fusion is present the proximity of said two labeling/visualization means become relatively close in an interphase and/or metaphase chromosomal spread.

17. A method according to Claim 14 wherein said nucleic probes have a complexity of from about 50 kilobases (kb) to about 1 megabase (Mb).

18. A method according to Claim 17 wherein the complexity is from about 50 kb to about 750 kb.

19. A method accoridng to Claim 18 wherein the complexity is from about 200 kb to about 400 kb.

20. A method according to Claim 9 wherein the genetic rearrangement occurs relatively closely in a genome to the location of another genetic rearrangement known to be associated with cancer.

21. A method according to Claim 20 wherein said nucleic acid probes produce staining patterns which distinguish a genetic rearrangement associated with either chronic myelogenous leukemia (CML) or acute lymphocytic leukemia (ALL) occurs.

22. Nucleic acid probes that reliably stain targeted chromosomal materials wherein said targeted chromosomal materials are in the vicinity of one or more suspected genetic rearrangements.

23. Nucleic acid probes according to Claim 22 that are appropriate for in situ hybridization.

24. Nucleic acid probes according to Claim 23 wherein said nucleic acid sequences are of sufficient complexity to stain reliably each of two or more target sites on chromosomal material in a genome.

25. Nucleic acid probes according to Claim 24 which are substantially free of nucleic acid sequences having hybridization capacity to sites on non-targeted chromosomal material.

26. Nucleic acid probes according to Claim 22 wherein said one or more genetic rearrangements is or are selected from the group consisting of translocations, inversions, insertions, amplifications and deletions.

27. Nucleic acid probes according to Claim 22 wherein said one or more genetic rearrangements is or are associated with one or more diseases.

28. Nucleic acid probes according to Claim 27 wherein said one or more genetic rearrangements is or are associated with cancer.

29. Nucleic acid probes according to Claim 28 wherein said one or more genetic rearrangements occurs or occur relatively closely in a genome to another genetic rearrangement known to be associated with cancer.

30. Nucleic acid probes according to Claim 29 wherein said genetic rearrangements are associated with either CML and/or ALL.

31. Nucleic acid probes according to Claim 29 wherein said probes produce a staining pattern which distinguish genetic rearrangements associated with either CML or ALL occurs.

32. High complexity nucleic acid probes for the detection of genetic rearrangements.

33. High complexity nucleic acid probes according to Claim 32 wherein the complexity is greater than 50,000 bases.

34. High complexity nucleic acid probes according to Claim 32 wherein the genetic rearrangements are selected from the group consisting of translocations, inversions, insertions, amplifications and deletions.

35. High complexity nucleic acid probes according to Claim 34 wherein said genetic rearrangements are associated with cancer.

36. High complexity nucleic acid probes according to Claim 35 wherein said genetic rearrangements are diagnostic for CML.

37. High complexity nucleic acid probes according to Claim 36 wherein said genetic rearrangements are selected from the group consisting of translocations, insertions and amplifications.

38. High complexity nucleic acid probes according to Claim 37 which produce a staining pattern which is distinctively altered when the BCR-ABL fusion characteristic of CML occurs.

39. High complexity nucleic acid probes according to Claim 38 which have a complexity of from about 50 kb to about 1 megabase.

40. High complexity nucleic acid probes according to Claim 39 wherein the complexity is from about 50 kb to about 750 kb.

41. High complexity nucleic acid probes according to Claim 40 wherein the complexity is from about 200 kb to about 400 kb.

42. A method of detecting genetic rearrangements comprising the steps of:

a. hybridizing the probes of Claim 32 to targeted chromosomal material in the vicinity of a suspected genetic rearrangement;

b. observing and/or measuring the proximity of and/or other characteristics of the signals from said probes; and

c. determining from said observations and/or measurements obtained in step b) whether a genetic rearrangement has occurred.

43. A method according to Claim 42 wherein the suspected genetic rearrangement is associated with cancer.

44. A method according to Claim 43 wherein the cancer is CML or ALL.

45. A method according to Claim 44 wherein the cancer is CML and the genetic rearrangement produces a BCR-ABL fusion.

46. A method according to Claim 42 wherein the chromosomal material is either in metaphase or in interphase.

47. A method according to Claim 46 wherein the chromosomal material is in metaphase.

48. A method according to Claim 46 wherein the chromosomal material is in interphase.

49. A method according to Claim 43 wherein the suspected genetic rearrangement is prognostic of cancer.

50. A method according to Claim 49 wherein the cancer is CML.

51. A method according to Claim 50 wherein staining patterns produced therefrom are used to distinguish normal and malignant cells for purposes of prognosis and/or determining the effectiveness of therapy.

52. A method according to Claim 51 wherein said therapeutic regimens are selected from the group consisting of chemotherapy, radiation, surgery and transplantation.

53. A method according to Claim 50 wherein staining patterns produced therefrom are useful in monitoring the status of a patient whose chromosomal material is so tested on a cell to cell basis.

54. A method of determining the molecular basis of genetic disease employing the probes of Claim 32.

55. A method of distinguishing between CML and ALL based upon staining patterns produced by the method of Claim 44.

56. A method according to Claim 53 wherein the patient is in remission and staining patterns produced therefrom are predictive of a recurrence of cancer.

57. High complexity nucleic acid probes according to Claim 52 wherein the targeted chromosomal material is human and selected from DNA of chromosomes 1 through 22, X and Y.

58. High complexity nucleic acid probes according to Claim 32 wherein the probe nucleic acid sequences are propagated in a cell line and/or in one or more vectors.

59. High complexity nucleic acid probes according to Claim 58 wherein said cell line is a hybrid cell line and said one or more vectors is or are selected from the group consisting of yeast artificial chromosomes, plasmids, bacteriophages and cosmids.

60. A method of staining targeted chromosomal material in the vicinity of a suspected genetic rearrangement with high complexity nucleic acid probes according to Claim 32 wherein the probe nucleic acid sequences

prior to hybridization to the targeted chromosomal material are broken into fragments of from about 200 bases to about 2000 bases.

61. A method according to Claim 60 wherein the size of the fragments are about 1 kb.

62. A method according to Claim 61 wherein the size of the fragments is from about 800 bases to about 1000 bases and, wherein the hybridization is performed at a temperature of about 30 degrees C to about 45 degrees C, and wherein the subsequent washing steps are performed at a temperature of from about 40 degrees C to about 50 degrees C.

63. A method according to Claim 62 wherein the hybridization is performed at a temperature of from about 35 degrees C to about 40 degrees C.

64. A method according to Claim 63 wherein the hybridization is performed at a temperature of about 37 degrees C, and the subsequent washing steps are performed at a temperature of about 45 degrees C.

65. A method according to Claim 61 wherein the labeled fragments are detected after hybridization by flow cytometry.

66. A method according to Claim 60 wherein detection is by microscopy.

67. A method according to Claim 66 wherein the microscopy is automated.

68. A method according to Claim 65 wherein light scattering is used.

69. High complexity probes according to Claim 32 wherein the targeted chromosomal material of said probes is chromosomal material of fetal cells.

70. High complexity probes according to Claim 69 wherein said fetal cells have been separated from maternal blood.

71. High complexity probes according to Claim 32 wherein the targeted chromosomal material is in interphase and/or metaphase.

72. Chromosome-specific staining reagent comprising a heterogeneous mixture of labeled nucleic acid fragments, wherein the labeled nucleic acid fragments are complementary to sites on targeted chromosomal material in the vicinity of suspected genetic rearrangements and are substantially free of nucleic acid sequences having hybridization capacity to sites on non-targeted chromosomal material.

73. The chromosome-specific staining reagent of Claim 72 wherein said labeled nucleic acid fragments are single-stranded.

74. The chromosome-specific staining reagent of Claim 73 wherein said nucleic acid fragments are labeled with radioactive, enzymatic, immunoreactive, fluorochromes and/or affinity detectable reagents.

75. The chromosome-specific staining reagent of Claim 74 wherein said fragments are biotinylated, modified with N-acetoxy-N-2-acetylaminofluorene, modified with fluorescein isothiocyanate, modified with mercury/TNP ligand, sulfonated, digoxigenenated, or contain T-T dimers.

76. A chromosome-specific staining reagent that provides staining patterns indicative of a genetic rearrangement produced by the process of:

isolating chromosome-specific DNA;

amplifying pieces of the isolated chromosome-specific DNA;

disabling the hybridization capacity of and/or removing shared repetitive sequences contained in the amplified pieces of the isolated DNA to form a collection of nucleic acid fragments which hybridize predominantly to targeted chromosomal DNA in the vicinity of a suspected genetic rearrangement; and

labeling the nucleic acid fragments of the collection to form a heterogeneous mixture of nucleic acid fragments.

77. A chromosome-specific staining reagent according to Claim 76 wherein said step of amplifying said pieces of isolated DNA is performed by cloning.

78. A chromosome-specific staining reagent according to Claim 76 wherein said step of amplifying said pieces of isolated DNA is performed by using the polymerase chain reaction (PCR).

79. The chromosome-specific staining reagent of Claim 76 wherein said step of removing said shared repetitive sequences comprises selecting amplified pieces of said isolated DNA which pieces are substantially free of nucleic acid sequences which are complementary to non-targeted chromosomal material.

80. The chromosome-specific staining reagent of Claim 79 wherein said selection of said amplified pieces comprises the use of Southern hybridization.

81. The chromosome-specific staining reagent of Claim 80 wherein said selection of said amplified pieces comprises screening clones for the presence of repetitive sequences by hybridization with genomic DNA.

82. The chromosome-specific staining reagent of Claim 81 wherein said clones are plasmid clones.

83. The chromosome-specific staining reagent of Claim 82 wherein said selection of said amplified pieces comprises screening said clones for hybridization to the targeted chromosomal material, and removing the clones which do not so hybridize.

84. The chromosome-specific staining reagent of Claim 76 wherein said step of disabling said hybridization capacity comprises hybridizing said amplified pieces of said isolated DNA with unlabeled nucleic acid sequences.

85. The chromosome-specific staining reagent of Claim 84 wherein said step of disabling said hybridization capacity comprises the addition of unlabeled blocking nucleic acid to the labeled nucleic acid probe prior to and/or during hybridization to the targeted chromosomal material.

86. The chromosome-specific staining reagent of Claim 85 wherein the unlabeled blocking DNA is genomic.

87. The chromosome-specific staining reagent of Claim 86 wherein the blocking nucleic acid is a high-copy fraction of genomic DNA.

88. The chromosome-specific staining reagent of Claim 85 wherein the unlabeled blocking DNA is from a selection of clones containing the highest copy sequences from a genome and/or additional clones as required to produce useful contrast.

89. The chromosome-specific staining reagent of Claim 76 wherein said step of disabling said hybridization capacity of said shared repetitive sequences comprises self-reassociating the high complexity probe.

90. The chromosome-specific staining reagent of Claim 76 wherein said step of removing the shared repetitive sequences comprises the use of hydroxyapatite chromatography.

91. The chromosome-specific staining reagent of Claim 76 wherein said step of removing the shared repetitive sequences comprises reacting the amplified pieces of the isolated DNA with immobilized, single-stranded nucleic acid sequences which are complementary to said shared repetitive sequences.

92. A method of staining targeted chromosomal material with a high complexity nucleic acid probes according to Claim 32 to produce staining patterns indicative of genetic rearrangements wherein unlabeled high copy repetitive nucleic acid sequences or genomic DNA are hybridized to the targeted chromosomal material prior to or during the hybridization with the high complexity nucleic acid probe.

93. A method of staining targeted chromosomal material with high complexity nucleic acid probes according to Claim 32 to produce staining patterns indicative of genetic rearrangements comprising the steps of:

providing a heterogeneous mixture of labeled nucleic acid fragements, wherein substantial proportions of the labeled nucleic acid fragments in the heterogeneous mixture have base sequences substantially complementary to the targeted chromosomal material which is in the vicinity of a suspected genetic rearrangement;

reacting the heterogeneous mixture with the targeted chromosomal DNA by in situ hybridization; and

observing and/or measuring the proximity of and/or other characteristics of signals of said staining patterns to determine whether a genetic rearrangement has occurred.

94. High complexity nucleic acid probes according to Claim 32 which are substantially free of shared repetitive sequences produced by a process employing a polymerase chain reaction (PCR) procedure.

95. High complexity nucleic acid probes according to Claim 94 wherein during said PCR process, sequences which are complementary to said shared repetitive sequences, and which have extended non-complementary ends or which are terminated in nucleotides which do not permit extension by a polymerase, are hybridized to said shared repetitive sequences to inhibit amplification of such sequences.

96. A method of staining targeted chromosomal material in the vicinity of a suspected genetic rearrangement with high complexity nucleic acid probes of Claim 32 wherein the probes are not directly labeled and detection of the probes bound to the targeted chromosomal material is by means other than direct labeling.

97. A method of staining targeted chromosomal material according to Claim 96 wherein the means of detecting the probes bound to the targeted

chromosomal material comprise the use of anti-RNA/DNA duplex antibodies and/or anti-thymidine dimer antibodies.

98. High complexity nucleic acid probes according to Claim 32 for detection of specific genetically based diseases.

99. High complexity nucleic acid probes according to Claim 32 for detection of genetic rearrangements induced by exposure to clastogenic agents.

100. High complexity nucleic acid probes according to Claim 99 wherein the probes have been optimized for rapid detection of structural chromosome aberrations.

101. High complexity nucleic acid probes according to Claim 32 wherein said genetic rearrangements are indicative of cytogenetic abnormalities in tumor cells.

102. Test kits comprising the probes of Claim 32.

103. Test kits comprising the probes of Claim 38.

104. High complexity nucleic acid probes according to Claim 32 for use in biological dosimetry.

105. A method of distinguishing between suspected genetic rearrangements that occur in relatively close proximity in a genome

comprising in situ hybridization with nucleic acid probes which comprise sequences which are substantially homologous to nucleic acid sequences in the vicinity of said suspected genetic rearrangements.

106. A method according to Claim 105 wherein said suspected genetic rearrangements are associated with a disease.

107. A method according to Claim 106 wherein the disease is cancer.

108. A method according to Claim 107 wherein said suspected genetic rearrangements are associated with CML and ALL.

109. A method of detecting a contiguous gene syndrome comprising the in situ hybridization of nucleic acid probes that comprise sequences which are substantially homologous to nucleic acid sequences characteristic of one or more components of said contiguous gene syndrome.

110. A method according to Claim 109 wherein said contiguous gene syndrome is Down syndrome.

111. Nucleic acid probes, according to Claim 32, comprising nucleic acid sequences that are substantially homologous to nucleic acid sequences in chromosomal regions that flank and/or extend partially or fully across breakpoints associated with cytogenetically similar but genetically different diseases.

112. A method of distinguishing cytogenetically similar but genetically different diseases comprising the in situ hybridization of the probes of Claim 111 to metaphase and/or interphase chromosomal spreads.

113. The method of Claim 112 wherein the chromosomal spreads are human.

114. Nucleic acid probes according to Claim 111 wherein the diseases are cancer.

115. Nucleic acid probes according to Claim 114 wherein the diseases are CML and ALL.

116. A chromosome-specific staining reagent according to Claim 72 which is disease-specific.

117. A chromosome-specific staining reagent according to Claim 116 which is tumor-specific.

118. A chromosome-specific staining reagent according to Claim 17 which is specific for the BCR-ABL fusion characteristic of CML.

119. A chromosome-specific staining regent according to Claim 76 which is disease specific.

120. High complexity nucleic acid probes according to Claim 32 comprising nucleic acid sequences substantially homologous to multiple loci in a genome.

121. High complexity nucleic acid probes according to Claim 120 wherein said sequences are associated with regions of the genome in which genetic rearrangements are known to occur.

122. High complexity nucleic acid probes according to Claim 121 wherein the genome is human.

123. A method of simultaneously detecting the genetic rearrangements of multiple loci in a genome comprising in situ hybridization with the probes of Claim 120.

124. A method of searching for a genetic rearrangement in a chromosomal region of a genome indicated by conventional banding analysis to contain an abnormality comprising in situ hybridization of nucleic acid probes according to Claim 32.

125. A method according to Claim 53 wherein computer assisted microscopic analysis is used to search for any residual disease in said patient.

126. High complexity nucleic acid probes according to Claim 32 wherein the probes have been optimized for rapid, efficient, automated detection of aberrations.